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(54) Title: ISOLATION AND CHARACTERIZATION OF cDNA OF *PLASMODIUM FALCIPARUM* GLUCOSE-6-PHOSPHATE DEHYDROGENASE

(57) Abstract

DNA segments encoding the *Plasmodium falciparum* glucose-6-phosphate dehydrogenase protein obtained by polymerase chain reaction techniques.

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ISOLATION AND CHARACTERIZATION OF cDNA OF *PLASMODIUM FALCIPARUM* GLUCOSE-6-PHOSPHATE DEHYDROGENASE

1. Field of the Invention

The present invention relates to glucose-6-phosphate dehydrogenase from *Plasmodium falciparum* and to the DNA segment which encodes it.

2. Background Information

Glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme in the pentose phosphate pathway. In most organisms the pathway has two main functions: production of pentose (ribose) for biosynthesis of nucleic acids and several coenzymes, and reduction of NADP for a variety of detoxification and reductive biosynthetic reactions. Recently, Vander Jagt et al. reported that isocitrate dehydrogenase may be responsible for providing much of the NADPH required for reductive biosynthesis within the *Plasmodium falciparum* parasite (D.L. Vander Jagt, L.A. Hunsaker, M. Kibirige, N.M. Campos, Blood, 74, 1, 471-474 (1989)); while, Roth et al. reported that the majority of ribose synthesis in parasite infected red blood cells (RBCs) appears to occur through pathways other than those involving G6PD (E.F. Roth, R.M. Ruprecht, S. Schulman, J. Vanderberg, J.A. Olson, J. Clin. Invest., 77, 1129-1135 (1986)). Therefore, consistent with the findings of Usanga and Luzzatto, parasite encoded G6PD does not seem necessary for parasite survival in normal erythrocytes (RBCs) (E.A. Usanga, L. Luzzatto, Nature, 313, 793-795 (1985)).

Several investigators have reported that when cultured in G6PD deficient RBCs, *P. falciparum*

parasites initially have a reduced growth rate, but following an adaptation period, the growth again approximates in vivo rates (Usanga et al. (1985); I.T. Ling, R.J.M. Wilson, Mol. & Biochem. Parasit., 31, 47-56 (1988)); E.F. Roth, C. Raventos-Suarez, A. Rinaldi, R.L. Nagel, PNAS, 80, 298-299 (1983)); and E.F. Roth, S. Schulman, Brit. J. Hema., 70, 363-367 (1988). Production of parasite G6PD following a lag phase seems to fully explain the recovery of normal growth rate during persistent culture in G6PD deficient erythrocytes (Usanga et al. (1985)). However, it has been subsequently observed (Ling et al. (1988); Roth et al. (1983); Roth et al. (1988); and B. Kurdi-Haidar, L. Luzzatto, Mol. & Biochem. Parasit., 41, 83-92 (1990)) that the parasite expresses G6PD constitutively, even in G6PD normal RBCs. The mechanism by which the parasite recovers to normal growth within a few cell cycles in G6PD deficient RBCs, and the mechanism that confers relative protection against malaria in females heterozygous for G6PD deficiency, despite expression of parasite encoded G6PD, now remain an even more perplexing enigma.

Further characterization and subcellular localization of the parasite encoded G6PD may provide clues as to how the parasite adapts in homozygous or hemizygous G6PD deficient erythrocytes, yet apparently fails to adapt in female mosaic. Such further characterization and localization may also lead to a new class of chemotherapeutic agents effective against the ever increasing population of drug resistant malaria parasites. To this end the *P. falciparum* glucose-6-phosphate dehydrogenase gene has been isolated and sequenced (and expressed in *Escherichia coli*).

Given the strong genetic and epidemiological evidence linking human G6PD deficiency with protection from malaria, and widespread resistance to current chemotherapeutic agents, development of a new class of agents directed against the potential "achilles heel" of the parasite was the impetus for the research that lead to the cloning of G6PD.

SUMMARY OF THE INVENTION

It is an object of the present invention to characterize the molecular structure of the glucose-6-phosphate dehydrogenase enzyme of *Plasmodium falciparum* in order to better design and exploit chemotherapeutic agents against malaria.

Accordingly, the present invention relates to DNA segments encoding glucose-6-phosphate dehydrogenase in *Plasmodium falciparum*.

The present invention additionally relates to the amino acid sequence of *Plasmodium falciparum* glucose-6-phosphate dehydrogenase.

Various other objects and advantages of the present invention will become obvious from the figure and the following description of the invention.

All publications mentioned herein are hereby incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) of the cDNA encoding *Plasmodium falciparum* glucose-6-phosphate dehydrogenase protein.

Figure 2 shows the deduced amino acid sequence (SEQ ID NO:2) of the protein encoded by *the cDNA of Figure 1.*

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a cDNA clone isolated by polymerase chain reaction techniques which encodes the glucose-6-phosphate dehydrogenase protein from *Plasmodium falciparum*. The isolated cDNA clone can be obtained in a substantially pure form by using conventional methods used by those of ordinary skill in the art.

The present invention also relates to the glucose-6-phosphate dehydrogenase protein from *Plasmodium falciparum* encoded by the cDNA. The protein has a novel structure as compared to all other (human, rat, fruit fly, yeast, and *E. coli*) G6PD deduced amino acid sequences. Although the predicted NADP binding site and glucose-6-phosphate binding site is conserved, the *P. falciparum* enzyme apparently has a secretory signal sequence, a membrane spanning segment, and a transmembrane helix, none of which are found in other G6PD deduced amino acid sequences.

The present invention further relates to a recombinantly produced *P. falciparum* G6PD protein with the amino acid sequence given in Figure 1, plus any allelic and/or biologically functioning variants of this sequence, or any unique portion of this sequence. The recombinant protein can be expressed in a number of expression systems, including both bacterial and eukaryotic. Further, the present invention relates to a synthetic *P. falciparum* G6PD protein.

The present invention relates to a recombinant DNA molecule comprising a vector and a DNA segment encoding the *P. falciparum* G6PD protein. Using methodology well known in the art, recombinant DNA molecules of the present invention can be constructed. Possible vectors for use in the present invention include, but are not limited to pUC 13, pUC 19, pcDNAII, pBluescriptII. The DNA segment can be present in the vector operably linked to regulatory elements, including, for example, a promoter.

The invention further relates to host cells comprising the above-described recombinant DNA molecule. The recombinant DNA molecule may be stably transformed, stably transfected, transiently transfected into the host cell or in alive attenuated virus. In each case, the host cell expresses a functionally active form of the protein encoded by the recombinant DNA molecule. The host cells used can be either bacterial or eukaryotic. Some non-limiting examples of bacterial host cells are *Escherichia coli* and *Staphylococcus aureus*. Non-limiting examples of eukaryotic host cells are *Saccharomyces cerevisiae*, CHO cells, COS cells, and Sf9 cells. Transformation with the recombinant molecules can be effected using methods well known in the art.

The present invention further relates to a method of screening drugs for anti-malarial activity by contacting a drug to the recombinant *P. falciparum* G6PD protein under conditions such that inhibition of said *P. falciparum* G6PD activity can be effected. (See D.C. Kaslow and S. Hill, JBC, 265, 21, 12337-12341, 1990.) By means of such drug screeing assays, the striking structural

features of the amino acid sequence of the protein can be exploited in the design of a chemotherapeutic intervention for malaria. The strong genetic and epidemiological evidence that human G6PD deficiency affords protection against malaria further suggests that malaria parasite G6PD may be a rational target for drug therapy.

Comparative assays were conducted to determine G6PD activity in the transfected cells which had been contacted with a drug versus G6PD activity in uncontacted transfected cells. After being contacted with the drug, the cells were placed in an environment where labeled glucose was the only source of carbon. Comparative assays were also conducted with untransfected cells as a control. The effect of the drug on the transfected cells was detected by measuring the presence of labelled PfG6PD reaction product. (Please correct and/or add further details to this Paper Example.)

The present invention further relates to antibodies specific for the *P. falciparum* G6PD protein of the present invention. One skilled in the art, using standard methodology, can raise antibodies (such as monoclonal, polyclonal, anti-idotypic and monoclonal catalytic [Sastry et al. PNAS 86:5728-5732 (1989)]) to the *P. falciparum* G6PD protein, or a unique portion thereof. In a further embodiment, such antibodies can be used in assays to detect the presence of *P. falciparum* G6PD protein in serum from a patient suspected of being infected with *P. falciparum*. Antibodies specific for the *P. falciparum* G6PD protein or a unique portion thereof can be coated on to a solid surface such as a plastic and contacted with the serum sample. After washing, the presence or absence of the protein from

the serum bound to the fixed antibodies is deteted by addition of a labeled (e.g. fluorescently labeled) antibody specific for the *P. falciparum* G6PD protein.

One skilled in the art will appreciate that the invention includes the use of competition type assays in detecting in a sample the antigens to which this invention relates.

The present invention also relates to a vaccine for use in humans against malaria. As is customary for vaccines, the *P. falciparum* G6PD protein, or a unique portion thereof, can be delivered to a human in a pharmacologically acceptable vehicle. As one skilled in the art will understand, it is not necessary to use the entire protein (for example, a synthetic polypeptide corresponding to the *P. falciparum* G6PD protein) can be used. Pharmacologically acceptable carriers commonly used in vaccines can be used to deliver the protein or peptide. Such carriers include MTP, tetanus toxoid or liposomes. Vaccines of the present invention can include effective amounts of immunological adjuvants known to enhance an immune response. Such adjuvants include IL-2 and alum.

The protein or polypeptide is present in the vaccine in an amount sufficient to induce an immune response against the antigenic protein and thus to protect against *Plasmodium* infection thereby protecting the human against malaria. Protective antibodies are usually best elicited by a series of 2-3 doses given about 1 to 6 months apart. The series can be repeated when concentrations of circulating antibodies in the human drops. Further, the vaccine can be used to immunize a human against

other forms of malaria (that is, heterologous immunization).

EXAMPLES

For purposes of illustrating a preferred embodiment of the present invention the following non-limiting examples will be discussed in detail.

Parasites and cDNA Library Construction.

The 3D7 clone of *P. falciparum* isolate NF54 (D. Walliker, I.A. Quakyi, T.E. Wellems, McCutchan, A. Szarfman, W.T. London, L.M. Corcoran, T.R. Burkot, R. Carter Science 236, 1661-1666 (1987)) and the HB3 isolate (Walliker et al. (1987)) were cultured in vitro. Total cellular RNA, purified from stage III to IV 3D7 gametocytes and from HB3 asexual parasites, was used to construct oligo dT primed, size-selected, BstXI linkered cDNA libraries in plasmid pcDNA II (Invitrogen). The libraries were screened (J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, 2d Ed. (1989)) with radiolabelled, random primed DNA probes (A.P. Feinberg, B. Vogelstein, Anal. Biochem. 137, 266-267 (1984)).

Polymerase Chain Reaction

Degenerate synthetic oligonucleotides were used to amplify the G6PD gene from *P. falciparum* cDNA or genomic DNA as follows: a sense strand oligonucleotide,

5'-ggaattcAT{ACT}GA{CT}CA{CT}TA{CT}
{CT}T{ACGT}GG{ACGT}AA{AG}GA-3',

located 5' of an antisense strand oligonucleotide,

5'-cggatccTG{AG}TT{TC}TGCAT{ACGT}
AC{AG}TC{ACGT}C-3',

were paired as primers in a polymerase chain reaction (R.K. Saiki, D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, H.A. Erlich, Science, 239, 487-491 (1988)). 4 cycles of denaturation at 94°C for 2 minutes, annealing at 37°C for 2 minutes, and extension at 72°C for 1 minute were followed by 25 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 30 seconds, and extension at 72°C for 1 minute; amplified DNA was purified and cloned as previously described (Kaslow et al. (1990)).

Northern and Southern Blots

Pulsed field gel electrophoresis was performed as described by Wellems et al. (T.E. Wellems, D. Walliker, C.L. Smith, V.E. Do Rosario, W.L. Maloy, R.J. Howard, R. Carter, T.F. McCutchan Cell 49, 633-642 (1987). Southern and Northern blot analyses was performed as described by Kaslow et al. (D.C. Kaslow, B.R. Migeon, M.G. Persico, M. Zollo, J.L. Vander Berg, P.B. Samollow, Genomics 1, 19-28 (1987)).

Cloning the PfG6PD Gene

Attempts to clone the *P. falciparum* G6PD gene by hybridization with human G6PD cDNA at low stringency or with "guessmers" comprising highly conserved regions, or by complementation in *pgi/zwf* deficient *E. coli* (DF214) either on glucose minimal media or on diamide containing rich media have been unsuccessful. Recently, the *Saccharomyces cerevisiae* G6PD gene was cloned: Thomas et al. cloned the gene by complementation for a defect in inorganic sulfur metabolism (methionine auxotrophy) (D. Thomas, H. Cherest, Y. Surdin-Kerjan, EMBO 10,

547-553 (1991)). *S. cerevisiae* G6PD gene was also cloned by using the polymerase chain reaction (PCR) with highly degenerate oligonucleotides (I. Nogae, M. Johnston, Gene, 96, 161-169 (1990).

When 6 sense and 11 antisense primers were used in PCR, only a single pair of primers was found to yield a fragment of the yeast gene. When this latter pair of primers was used in PCR with genomic yeast DNA or genomic *P. falciparum* DNA, a product was observed only in the reaction containing yeast DNA template. A further 13 permutations with 9 primers were examined by PCR using *P. falciparum* DNA as the template. One pair of primers (FIG. 1) amplified a 193bp fragment from *P. falciparum* DNA. The nucleotide sequence of this fragment differed from the published DNA sequences of human, *E. coli*, and *S. cerevisiae G6PD*, but typical of *P. falciparum* nucleotide sequence, was 74% A+T. In contrast, the deduced amino acid sequence from the fragment showed striking homology to mammalian, yeast, fruit fly, and bacterial G6PD amino acid sequence (FIG. 1).

P. falciparum gametocytes express parasite encoded G6PD at a high level. Therefore, to clone G6PD cDNA, a gametocyte specific cDNA library constructed in pcDNAII (Invitrogen) was screened with the 193bp PCR product. pPfg6pd2 (wpMS2) was selected for further characterization, and was found to have a 1750 bp insert, but did not contain the full length coding sequence (FIG. 1). An asexual stage cDNA library was also screened from which several additional clones were isolated. pPfg6pd6 (MS6) contained the most 5' sequence.

The insert from pPfg6pd2 hybridized to chromosome 14 by Southern blot analysis of size-fractionated *P. falciparum* chromosomes,

confirming that the cDNA originated from *P. falciparum* and not human RNA or other potential contaminants.

Sequence Analysis of pfG6PD

Universal sequencing primers and synthetic oligonucleotides are used to obtain DNA sequence from double stranded plasmid with Sequenase (United States Biochemicals Corp.). 100% of the sequence was determined from both strands.

A 2259 bp open reading frame, encoding an 88 kDa polypeptide of 751 amino acids, was deduced from the nucleotide sequence (FIG. 1). The presumptive initiation codon is in accordance with the *P. falciparum* consensus sequence, and the A+T content of 77% in the predicted coding region, and 85% in the 3' noncoding regions are typical of *P. falciparum* genes.

Comparison of the cDNA nucleotide sequence with that obtained from cloned genomic restriction enzyme fragments (nucleotide 562-1396), and comparison of PCR products from genomic DNA to that from cDNA suggest that the gene does not contain introns within this region but rather an insertion of 61 amino acids (residues 268-254) in between residues 111-137 of human G6PD (B. Persson, H. Jörnvall, I. Wood, J. Jeffery, FEBS, 1991, 486-491 (1991). Comparison of the deduced amino acid sequence with previously published human G6PD sequences revealed an overall identity of 39%.

The gene encoding *P. falciparum* G6PD is the first to be isolated in the pentose phosphate pathway from *Plasmodia*. As the genes encoding G6PD from mammals, insect, yeast, and bacteria have been sequenced, the structural similarities and

differences of the malaria parasite to other G6PD can be easily identified. For instance, the reactive lysyl residue in the predicted binding site for glucose-6-phosphate were identical in mammalian (human and rat), fruit fly, yeast, bacterial and parasite G6PD. The NADP binding site proposed by Beutler and colleagues based on convincing genetic evidence (A. Hirono, W. Kuhl, T. Gelbart, L. Forman, V.F. Fairbanks, E. Beutler, PNAS, 86, 10015-10017 (1989)) is not present in falciparum G6PD; however, the region proposed by Persson et al. based on recognizable characteristics of coenzyme binding sites, including GXXGXXA and ß-α-ß fold is present in the parasite deduced amino acid sequence. The surprising features of the predicted protein structure of the parasite G6PD enzyme, however, are its molecular mass, pI, and membrane associated motifs.

Pfg6pd, as compared to all of the other G6PD genes except *E. coli* that have been analyzed so far, has the least number of identical residues, and has a large insertion (residues 1-147) between the N-terminus and the putative NADP binding site and another large insertion (268-354) of 61 amino acids between that binding site and the G6P binding sites. These insertions make the predicted molecular mass of the monomer at least 82kDa rather than the 50-55kDa predicted for the other known G6PD enzymes. The N-terminal insertion contains two potentially important structures: a secretory signal sequence (residues 63-76) and a hydrophilic region (residues 123-135). The other insertion contains a potential transmembrane helical structure (residues 349-364) that the other G6PD proteins lack, despite the identity of a number of residues in this region.

Another membrane associated structure, a membrane spanning segment, is predicted toward the C-terminus (residues 614-630). Finally, the remarkably slow migration of *P. falciparum* G6PD in native PAGE may be explained by its predicted higher molecular mass.

Whether the unique features of *P. falciparum* G6PD target the enzyme to the endoplasmic reticulum for transport to the parasitophorous vacuole, or even to the RBC cytoplasm, or to another compartment within the parasite itself remain to be determined. Wherever the enzyme resides, the striking differences in the structure of G6PD between parasite and other organisms may potentially be exploited in the design of new chemotherapeutic agents against malaria.

* * * * * * * *

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Kaslow, David S.
Shahabuddin, Mohammed

(ii) TITLE OF INVENTION: Isolation and Characterization of cDNA of Plasmodium Falciparum Glucose-6-Phosphate Dehydrogenase

(iii) NUMBER OF SEQUENCES: 2

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(D) STATE: D.C.
(E) COUNTRY: U.S.A.
(F) ZIP: 20036-5601

(v) COMPUTER READABLE FORM:

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(B) COMPUTER: IBM PC compatible
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(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Scott, Watson T.
(B) REGISTRATION NUMBER: 26,581
(C) REFERENCE/DOCKET NUMBER: WTS/5683/92326/

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(C) TELEX: 6714627 CUSH

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 2750 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AAAATAATTA TATATATAAT GAAAAAACAT TAGATTTTAT AAATAATGAT CAAGATAATG   120

ATAATTTAAA ATATTTGAAA GAATATGTAT ATTTTACGAC AACAAATCAA TTTGATGTTA   180

GGAAAAGAAT TACAGTATCT TTAAATTTAT TAGCTAATGC ATCAAGTAAA ATATTTTTAT   240

TAAATTCTAA AGACAAATTA GATTTATGGA AAAATATGTT GATTAAATCA TATATTGAAG   300
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ATACCACTGT	TATATCTTGT	GGTTATGAAA	ATTATACAAA	ATATATTGAA	GAAATTTATG	480
ATTCTAAATA	TGCTCTATCT	CTTTATTCTA	ATAGTTTGAA	TAAAGAAGAA	TTATTAACTA	540
TAATAATTTT	TGGCTGTTCA	GGTGATTTAG	CCAAAAAAAA	AATATATCCA	GCTTTATTTA	600
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TTCAAGATTT	CGATACATTT	TTTGATAAAA	TAGTTATATA	TTTAAAACGA	TGTTTATTAT	720
GTTATGAAGA	TTGGTCTATA	TCAAAAAAGA	AGGATCTTTT	AAATGGTTTT	AAAAATAGGT	780
GTCGATATTT	TGTTGGTAAT	TATTCGTCTT	CAGAAAGTTT	TGAAAATTTT	AATAAATATT	840
TAACAACTAT	TGAAGAAGAA	GAAGCAAAAA	AAAAATATTA	TGCAACATGT	TATAAAATGA	900
ATGGTTCAGA	TTATAATATA	TCAAATAATG	TTGCAGAGGA	TAATATTAGT	ATAGATGATG	960

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AAAATAAGAC AAATGAATAT TTTCAAATGT GTACTCCAAA AAATTGCCCT GATAATGTAT   1020

TTTCATCAAA TTATAATTTT CCATATGTTA TAAATAGTAT ATTATATTTA GCATTACCTC   1080

CACATATATT TATTAGTACT TTAAAAAAAA TTATAAAAAA AAATTGTTTA AATAGTAAAG   1140

GCACTGATAA AATATTACTA GAAAAACCAT TTGGAAATGA TTTAGATTCA TTTAAAATGT   1200

TATCAAAACA AATATTAGAG AATTTTAATG AACAACAAAT ATATAGAATA GATCATTATT   1260

TGGGTAAGGA TATGGTTTCA GGATTGTTGA AATTAAAATT TACAAATACA TTTTTATTAT   1320

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TTTTTAAATC TGGAAAAGGT CTGAATAAAG ATATATGTGA AATACGTATA CAATTCCATA 1800

ATATTATGGG GTCGTCTGAT GAAAATATGA ATAATAATGA ATTTGTTATT ATATTACAAC 1860

CTGTTGAAGC TATATACCTA AAAATGATGA TTAAAAAAAC GGGTTGTGAA GAAATGGAAG 1920

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CTTTTTATGA AGACGATTTG TTAGATATTA ATTATTAATT GATATATGTA TATATTTAAA 2280

TTAACCAAAT TAACACCCAA TGAATATGAA AATAATATAT ATATATATAT ATATATTATA 2340

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CATATATATA TATATATATA TATTATTTCA CTTATCTGCC CACGAACTTT ATTTTTGTTT 2460

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CCCATGTTTA ACTAATAATA TTACAAATAG AACTCAAAAA AAAAAAAAAT AATTATACAA 2700

TGAATTAAAG CTTTTTAATA TATTTTTAAT GGTATCTCCA GACTTTAGAG 2750

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 751 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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1               5                   10                  15

Asn Asn Tyr Gln Asn Asn Tyr Ile Tyr Asn Glu Lys Thr Leu Asp Phe
            20                  25                  30

Ile Asn Asn Asp Gln Asp Asn Asp Asn Leu Lys Tyr Leu Lys Glu Tyr
        35                  40                  45

Val Tyr Phe Thr Thr Thr Asn Gln Phe Asp Val Arg Lys Arg Ile Thr
    50                  55                  60

Val Ser Leu Asn Leu Leu Ala Asn Ala Ser Ser Lys Ile Phe Leu Leu
65                  70                  75                  80
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Asn Ser Lys Asp Lys Leu Asp Leu Trp Lys Asn Met Leu Ile Lys Ser
                85                  90                  95

Tyr Ile Glu Val Asn Tyr Asn Leu Tyr Pro Ala Thr Tyr Leu Ile Asp
            100                 105                 110

Thr Ser Cys Thr Asn Glu Asn Val Asn Ile Asn Asn Asn Asn Asn Asn
        115                 120                 125

Asn Asn Lys Asn Lys Asn Asn Tyr Cys Tyr Ser Asn Thr Thr Val Ile
    130                 135                 140

Ser Cys Gly Tyr Glu Asn Tyr Thr Lys Tyr Ile Glu Glu Ile Tyr Asp
145                 150                 155                 160

Ser Lys Tyr Ala Leu Ser Leu Tyr Ser Asn Ser Leu Asn Lys Glu Glu
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Leu Leu Thr Ile Ile Ile Phe Gly Cys Ser Gly Asp Leu Ala Lys Lys
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Lys Ile Tyr Pro Ala Leu Phe Lys Leu Phe Cys Asn Asn Ser Leu Pro
        195                 200                 205

Lys Asp Leu Leu Ile Ile Gly Phe Ala Arg Thr Val Gln Asp Phe Asp
    210                 215                 220

Thr Phe Phe Asp Lys Ile Val Ile Tyr Leu Lys Arg Cys Leu Leu Cys
225                 230                 235                 240

Tyr Glu Asp Trp Ser Ile Ser Lys Lys Lys Asp Leu Leu Asn Gly Phe
                245                 250                 255

Lys Asn Arg Cys Arg Tyr Phe Val Gly Asn Tyr Ser Ser Ser Glu Ser
            260                 265                 270

Phe Glu Asn Phe Asn Lys Tyr Leu Thr Thr Ile Glu Glu Glu Glu Ala
        275                 280                 285

Lys Lys Lys Tyr Tyr Ala Thr Cys Tyr Lys Met Asn Gly Ser Asp Tyr
    290                 295                 300
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Asn Ile Ser Asn Asn Val Ala Glu Asp Asn Ile Ser Ile Asp Asp Glu
305                 310                 315                 320

Asn Lys Thr Asn Glu Tyr Phe Gln Met Cys Thr Pro Lys Asn Cys Pro
                325                 330                 335

Asp Asn Val Phe Ser Ser Asn Tyr Asn Phe Pro Tyr Val Ile Asn Ser
            340                 345                 350

Ile Leu Tyr Leu Ala Leu Pro Pro His Ile Phe Ile Ser Thr Leu Lys
        355                 360                 365

Lys Ile Ile Lys Lys Asn Cys Leu Asn Ser Lys Gly Thr Asp Lys Ile
    370                 375                 380

Leu Leu Glu Lys Pro Phe Gly Asn Asp Leu Asp Ser Phe Lys Met Leu
385                 390                 395                 400

Ser Lys Gln Ile Leu Glu Asn Phe Asn Glu Gln Gln Ile Tyr Arg Ile
                405                 410                 415
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Asp His Tyr Leu Gly Lys Asp Met Val Ser Gly Leu Leu Lys Leu Lys
            420                 425                 430

Phe Thr Asn Thr Phe Leu Leu Ser Leu Met Asn Arg His Phe Ile Lys
        435                 440                 445

Cys Ile Lys Ile Thr Leu Lys Glu Thr Lys Gly Val Tyr Gly Arg Gly
    450                 455                 460

Gln Tyr Phe Asp Pro Tyr Gly Ile Ile Arg Asp Val Met Gln Asn His
465                 470                 475                 480

Met Leu Gln Leu Leu Thr Leu Ile Thr Met Glu Asp Pro Ile Asp Leu
                485                 490                 495

Asn Asp Glu Ser Val Lys Asn Glu Lys Ile Lys Ile Leu Lys Ser Ile
            500                 505                 510

Pro Ser Ile Lys Leu Glu Asp Thr Ile Ile Gly Gln Tyr Glu Lys Ala
        515                 520                 525
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Glu Asn Phe Lys Glu Asp Glu Asn Asn Asp Asp Glu Ser Lys Lys Asn
    530                 535                 540

His Ser Tyr His Asp Asp Pro His Ile Asp Lys Asn Ser Ile Thr Pro
545                 550                 555                 560

Thr Phe Cys Thr Cys Ile Leu Tyr Ile Asn Ser Ile Asn Trp Tyr Gly
                565                 570                 575

Val Pro Ile Ile Phe Lys Ser Gly Lys Gly Leu Asn Lys Asp Ile Cys
            580                 585                 590

Glu Ile Arg Ile Gln Phe His Asn Ile Met Gly Ser Ser Asp Glu Asn
        595                 600                 605

Met Asn Asn Asn Glu Phe Val Ile Ile Leu Gln Pro Val Glu Ala Ile
    610                 615                 620

Tyr Leu Lys Met Met Ile Lys Lys Thr Gly Cys Glu Glu Met Glu Glu
625                 630                 635                 640
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Val Gln Leu Asn Leu Thr Val Asn Glu Lys Asn Lys Lys Ile Asn Val
                645                 650                 655

Pro Glu Ala Tyr Glu Thr Leu Leu Leu Glu Cys Phe Lys Gly His Lys
            660                 665                 670

Lys Lys Phe Ile Ser Asp Glu Glu Leu Tyr Glu Ser Trp Arg Ile Phe
        675                 680                 685

Thr Pro Leu Leu Lys Glu Leu Gln Glu Lys Gln Val Lys Pro Leu Lys
    690                 695                 700

Tyr Ser Phe Gly Ser Ser Gly Pro Lys Glu Val Phe Gly Leu Val Lys
705                 710                 715                 720

Lys Tyr Tyr Asn Tyr Gly Lys Asn Tyr Thr His Arg Pro Glu Phe Val
                725                 730                 735

Arg Lys Ser Ser Phe Tyr Glu Asp Asp Leu Leu Asp Ile Asn Tyr
            740                 745                 750
```

WHAT IS CLAIMED IS:

1. A purified DNA segment, wherein said segment has a nucleotide sequence or a unique portion of said sequence as shown in Fig. 1 (SEQ ID NO:1).

2. A protein, wherein said protein has an amino acid sequence or a unique portion of said sequence as shown in Fig. 2 (SEQ ID NO:2).

3. A DNA segment encoding the protein of claim 2.

4. The protein according to claim 2 separated from proteins with which said protein is naturally associated.

5. A recombinantly produced protein having at least a unique portion of the amino acid sequence given in Fig. 2 (SEQ ID NO:2).

6. A recombinant DNA molecule comprising:

i) said DNA segment according to claim 3; and

ii) a vector.

7. A host cell stably transfected with the recombinant DNA molecule of claim 6 in a manner allowing expression of a functionally active form of said protein encoded by said DNA molecule.

8. The host cell according to claim 7 which is *Escherichia coli*.

9. The host cell according to claim 7 which is a eukaryotic cell.

10. A method of producing a recombinant *Plasmodium falciparum* glucose-6-phosphate dehydrogenase protein comprising culturing said host cells according to claim 7, in a manner allowing expression of said protein and isolation of said protein.

11. A method of screening drugs for activity against *Plasmodium falciparum* glucose-6-phosphate dehydrogenase comprising the steps of:

i) contacting said drug to the host cell of claim 7,

ii) placing said drug-contacted host cell into an environment wherein all glucose is labelled glucose,

iii) detecting the presence or absence of a labelled reaction product of said labelled glucose and *Plasmodium falciparum* glucose-6-phosphate dehydrogenase; and

iv) performing appropriate control assays.

12. An antibody specific for the protein encoded by said DNA segment according to claim 1.

13. The antibody according to claim 12 which is polyclonal.

14. The antibody according to claim 12 which is monoclonal.

15. A bioassay for the diagnosis of *P. falciparum* infection comprising the steps of:

i) coating a surface with antibodies according to claim 12;

ii) contacting said coated surface with serum from a mammal suspected of infection with *P. falciparum*; and

iii) detecting the presence or absence of a complex formed between said antibodies and proteins present in the serum.

16. A vaccine against malaria comprising all, or a unique portion of a protein encoded by said DNA segment according to claim 1, in an amount sufficient to induce immunization against said disease, and a pharmaceutical carrier.

17. The vaccine according to claim 16 which further comprises an adjuvant.

FIG. 1A

Plasmodium falciparum Glucose-6-phosphate Dehydrogenase gene

PARTIAL SEQUENCE 2750 BP; 1164 A; 260 C; 323 G; 1003 T

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ATTTCCATAT TGCCAGTTTA TTTCCAAATA TATTTTATAA TATATATATG AATAACTATC
AAAATAATTA TATATATAAT GAAAAAACAT TAGATTTTAT AAATAATGAT CAAGATAATG
ATAATTTAAA ATATTTGAAA GAATATGTAT ATTTTACGAC AACAAATCAA TTTGATGTTA
GGAAAAGAAT TACAGTATCT TTAAATTTAT TAGCTAATGC ATCAAGTAAA ATATTTTTAT
TAAATTCTAA AGACAAATTA GATTTATGGA AAAATATGTT GATTAAATCA TATATTGAAG
TGAATTATAA TTTATATCCA GCTACTTATT TAATAGATAC ATCATGCACC AACGAAAATG
TTAATATTAA CAATAACAAC AATAATAATA ATAAGAATAA GAATAATTAT TGTTATAGTA
ATACCACTGT TATATCTTGT GGTTATGAAA ATTATACAAA ATATATTGAA GAAATTTATG
ATTCTAAATA TGCTCTATCT CTTTATTCTA ATAGTTTGAA TAAAGAAGAA TTATTAACTA
TAATAATTTT TGGCTGTTCA GGTGATTTAG CCAAAAAAAA AATATATCCA GCTTTATTTA
AATTATTTTG TAATAATTCC TTACCAAAAG ATTTATTAAT CATTGGATTT GCTAGAACAG
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FIG. 1B

```
TTCAAGATTT CGATACATTT TTTGATAAAA TAGTTATATA TTTAAAACGA TGTTTATTAT
GTTATGAAGA TTGGTCTATA TCAAAAAAGA AGGATCTTTT AAATGGTTTT AAAAATAGGT
GTCGATATTT TGTTGGTAAT TATTCGTCTT CAGAAAGTTT TGAAAATTTT AATAAATATT
TAACAACTAT TGAAGAAGAA GAAGCAAAAA AAAAATATTA TGCAACATGT TATAAAATGA
ATGGTTCAGA TTATAATATA TCAAATAATG TTGCAGAGGA TAATATTAGT ATAGATGATG
AAAATAAGAC AAATGAATAT TTTCAAATGT GTACTCCAAA AAATTGCCCT GATAATGTAT
TTTCATCAAA TTATAATTTT CCATATGTTA TAAATAGTAT ATTATATTTA GCATTACCTC
CACATATATT TATTAGTACT TTAAAAAAAA TTATAAAAAA AAATTGTTTA AATAGTAAAG
GCACTGATAA AATATTACTA GAAAAACCAT TTGGAAATGA TTTAGATTCA TTTAAAATGT
TATCAAAACA AATATTAGAG AATTTTAATG AACAACAAAT ATATAGAATA GATCATTATT
TGGGTAAGGA TATGGTTTCA GGATTGTTGA AATTAAAATT TACAAATACA TTTTTATTAT
CTTTAATGAA TAGACATTTT ATAAAATGTA TTAAAATTAC TCTTAAAGAA ACTAAAGGTG
TATATGGTAG AGGACAATAT TTTGATCCCT ATGGTATTAT TAGAGATGTT ATGCAAAATC
ATATGTTACA ATTATTAACA TTAATAACTA TGGAAGATCC TATAGATTTA AATGATGAAT
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FIG. 1C

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CTGTAAAAAA TGAGAAAATA AAAATTCTTA AATCAATTCC TTCGATCAAA TTAGAAGATA
CTATTATTGG ACAATATGAA AAAGCTGAAA ATTTTAAAGA AGATGAAAAT AATGATGATG
AATCGAAAAA AAATCATAGT TATCATGATG ATCCACATAT AGATAAAAAT TCGATTACTC
CAACATTTTG TACATGTATC TTATATATTA ATTCAATTAA TTGGTATGGT GTACCAATCA
TTTTTAAATC TGGAAAAGGT CTGAATAAAG ATATATGTGA AATACGTATA CAATTCCATA
ATATTATGGG GTCGTCTGAT GAAAATATGA ATAATAATGA ATTTGTTATT ATATTACAAC
CTGTTGAAGC TATATACCTA AAAATGATGA TTAAAAAAAC GGGTTGTGAA GAAATGGAAG
AAGTACAATT AAACCTAACA GTGAATGAGA AAAATAAAAA AATTAATGTA CCAGAAGCAT
ATGAAACATT ACTCTTAGAA TGTTTTAAAG GACATAAAAA AAAATTCATC TCAGACGAGG
AATTGTATGA ATCATGGAGA ATATTTACTC CTTTACTTAA GGAACTCCAG GAAAAACAAG
TCAAGCCTCT TAAATATTCT TTTGGATCAT CAGGCCCTAA AGAGGTATTT GGACTTGTCA
AAAAATATTA CAATTATGGT AAAAATTATA CGCACAGACC TGAGTTTGTT AGAAAATCCT
CTTTTTATGA AGACGATTTG TTAGATATTA ATTATTAATT GATATATGTA TATATTTAAA
TTAACCAAAT TAACACCCAA TGAATATGAA AATAATATAT ATATATATAT ATATATTATA
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FIG. ID

TGATTGTTTA GTATATTATT ACCTATCTTT TATAAGATAA CATAAATGTA TATATTATGA

CATATATATA TATATATATA TATTATTTCA CTTATCTGCC CACGAACTTT ATTTTTGTTT

TAAAATTCTA GTATAATTAA ATAAAAGAAA ATATTTGGAA CAATTTGCAT TTTTTATGTA

TAAATAAAAT TTATATAATA ATATACTTTC ATACTTACTT TTTATTTTAT TTTATTTTAT

TTATTTTTTA AATGTCTATT ATATATACAT ATAAATGCGT TTTCAAATAA ATAATAAAAA

CCCATGTTTA ACTAATAATA TTACAAATAG AACTCAAAAA AAAAAAAAAT AATTATACAA

TGAATTAAAG CTTTTTAATA TATTTTTAAT GGTATCTCCA GACTTTAGAG

FIG. 2

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ID PfG6PD      PRELIMINARY; PRT; 751 AA.
DT  04-SEP-1991 (CREATED BY PC/GENEPROGRAM TRANSL)
DE  GLUCOSE-6-PHOSPHATE DEHYDROGENASE
OS  PLASMODIUM FALCIPARUM
CC  TRANSLATED FROM DNA SEQUENCE PFG6PDGENE (BASES 3 TO 2255)
SQ  SEQUENCE  751 AA;  88199 MW; 2999141 CN:
    FHIASLFPNI FYNIYMNNYQ NNYIYNEKTL DFINNDQDND NLKYLKEYVY FTTTNQFDVR
    KRITVSLNLL ANASSKIFLL NSKDKLDLWK NMLIKSYIEV NYNLYPATYL IDTSCTNENV
    NINNNNNNNN KNKNNYCYSN TTVISCGYEN YTKYIEEIYD SKYALSLYSN SLNKEELLTI
    IIFGCSGDLA KKKIYPALFK LFCNNSLPKD LLIIGFARTV QDFDTFFDKI VIYLKRCLLC
    YEDWSISKKK DLLNGFKNRC RYFVGNYSSS ESFENFNKYL TTIEEEEAKK KYYATCYKMN
    GSDYNISNNV AEDNISIDDE NKTNEYFQMC TPKNCPDNVF SSNYNFPYVI NSILYLALPP
    HIFISTLKKI IKKNCLNSKG TDKILLEKPF GNDLDSFKML SKQILENFNE QQIYRIDHYL
    GKDMVSGLLK LKFTNTFLLS LMNRHFIKCI KITLKETKGV YGRGQYFDPY GIIRDVMQNH
    MLQLLTLITM EDPIDLNDES VKNEKIKILK SIPSIKLEDT IIGQYEKAEN FKEDENNDDE
    SKKNHSYHDD PHIDKNSITP TFCTCILYIN SINWYGVPII FKSGKGLNKD ICEIRIQFHN
    IMGSSDENMN NNEFVIILQP VEAIYLKMMI KKTGCEEMEE VQLNLTVNEK NKKINVPEAY
    ETLLLECFKG HKKKFISDEE LYESWRIFTP LLKELQEKQV KPLKYSFGSS GPKEVFGLVK
    KYYNYGKNYT HRPEFVRKSS FYEDDLLDIN Y
```

INTERNATIONAL SEARCH REPORT	International application No. PCT/US92/07807

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 3/00, 7/00, 13/00; A61K 35/16; C12P 21/02; C12N 15/00
US CL :435/70.21, 172.2; 436/501; 514/12; 530/350, 388.4, 389.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/70.21, 172.2; 436/501; 514/12; 530/350, 388.4, 389.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, APS
search terms: glucose-6-phosphate dehydrogenase, g6pd, kaslow, sgagabuddin, plasmodium falciparum

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOLECULAR and BIOCHEMICAL PARASITOLOGY, Volume 31, issued 1988, I.R. Ling et al., "Glucose-6-phosphate dehydrogenase activity of the malaria parasite Plasmodium falciparum", pages 47-51, see entire document.	1-17
Y	NATURE, Volume 304, issued 07 July 1983, F.E.G. Cox, "Cloning genes for antigens of Plasmodium falciparum", pages 13-14, see entire document.	1-17
Y	"CURRENT PROTOCOLS IN MOLECULAR BIOLOGY", published 1987, by Wiley and Sons, see pages 11.3-11.11.4, see entire document.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date		
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 NOVEMBER 1992	02 DEC 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DONALD E. ADAMS, PH.D.
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